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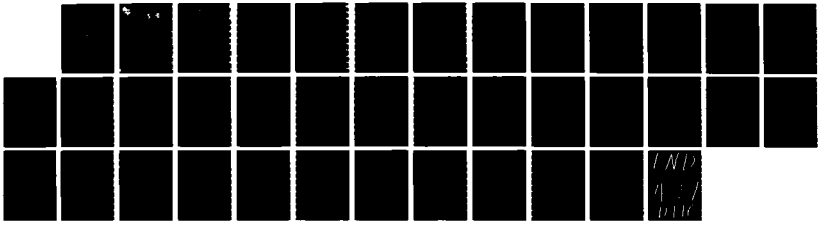

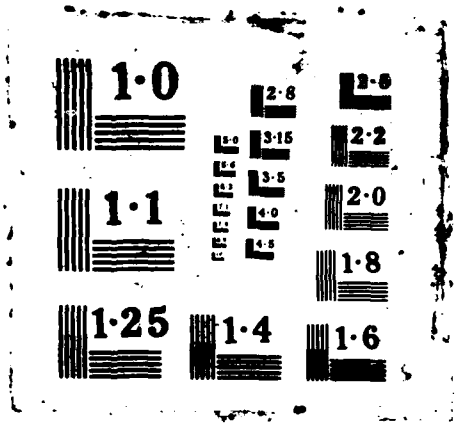
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HEMOGLOBIN SOLUTION EFFECTS ON THE HEART

Review of 19 Research Reports

D.H. MARKS, MD, PhD, MAJ USAF

DIVISION OF BLOOD RESEARCH

APRIL 1986

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This paper reviews 19 articles concerning the effect of hemoglobin solutions on the myocardium. Myocardial function can be supported in the presence of stroma-free hemoglobin solution with some beneficial and some detrimental effects. Most researchers have noted decreased cardiac work, decreased aortic and left ventricular filling pressure, decreased mean left atrial pressure, improved cardiac index and increased stroke work without a change in myocardial oxygen consumption. Most authors reported that

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21. ABSTRACT (Continued)

coronary vascular resistance (CVR) decreased and coronary blood flow (CBF) increased, although three papers noted the opposite (Kim et al, 1983; Dennis et al, 1983 and Suaudeau et al 1979). Biro (1982) even noted a decreased coronary sinus pO_2, implying an inadequate oxygen supply. In two papers, the researchers (Feola et al, 1979; Biro and Beresford-Kroeger, 1980) reported a decrease in myocardial infarct size as a result of SFHS infusion possibly as a result of the decreased CVR and increased CBF. This in itself has the potential for widespread use of SFHS.

Histologically Suaudeau et al (1978, 1979), but not Riedesel et al (1973) found that there was diffuse hemoglobin staining of myocardial tissue, extravasation of the hemoglobin, and cellular and interstitial edema. Vascular endothelial damage was observed (Suaudeau et al, 1979), as was subendocardial and midmural coagulation necrosis. Mitochondria were swollen, with loss of cristie and matrix granules. The addition of albumin to SFHS may counter these effects. High molecular weight polymers of SFHS were not studied. Much of the ultrastructural damage and some of the physiologic impairment could be related to an uncoupling of oxidative phosphorylation in the mitochondria, a known toxic effect of endotoxin. Perhaps this is the site of action for the known enhancement of endotoxin toxicity by hemoglobin. Hemoglobin itself might be toxic to mitochondria. It is also quite possible that the hemoglobin solutions used were contaminated with endotoxin, a known and significant problem. This possibility certainly would be worthy of further investigation.

The major problem with all these studies is the interpretation of data obtained in a nonphysiologic model. These animals were unconscious, intubated and ventilated, and had one to four drugs administered. In order to obtain meaningful data, hemoglobin solution which is endotoxin-free and physiologic with respect to electrolytes and oncotic pressure will need to be tested. The animal will need to be awake and unmedicated. Several models with these specifications exist and I hope they will be used in the near future.

ABSTRACT

This paper reviews 19 articles, obtained by an exhaustive search of the medline file of MEDLARS database, concerning the effect of hemoglobin solutions on the myocardium. Myocardial function can be supported in the presence of stroma-free hemoglobin solution with some beneficial and some detrimental effects. Most researchers have noted decreased cardiac work, decreased aortic and left ventricular filling pressure, decreased mean left atrial pressure, improved cardiac index and increased stroke work without a change in myocardial oxygen consumption. Most authors reported that coronary vascular resistance (CVR) decreased and coronary blood flow (CBF) increased, although three papers noted the opposite (Kim et al, 1983; Dennis et al, 1983 and Suaudeau et al 1979). Biro (1982) even noted a decreased coronary sinus pO_2, implying an inadequate oxygen supply. In two papers, the researchers (Feola et al, 1979; Biro and Beresford-Kroeger, 1980) reported a decrease in myocardial infarct size as a result of SFHS infusion possibly as a result of the decreased CVR and increased CBF. This in itself has the potential for widespread use of SFHS.

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Paper 4. Labrude P, Durelleroy M, Vigneron C. 1977. [The behaviour of haemoglobin solution in experimental circuits. Possible influence of the results on potential utilization in vivo.] Title Translated from the French. English Summary, Agressologie 18:21-27.

Paper 5. Biro GP, Beresford-Kroeger D, Smith BV. 1978. Myocardial oxygen-supply during hemodilution with stroma-free hemoglobin and methemoglobin solutions. In: Blood Substitutes and Plasma Expanders, [New York: Alan R Liss, Inc.] 213-222.

Paper 6. Biro GP, Beresford-Kroeger D. 1980. The effect of hemodilution with stroma-free hemoglobin and dextran on collateral perfusion of ischemic myocardium in the dog. Am Heart J 99:64-75.

Paper 7. Biro G. 1982. Comparison of acute cardiovascular effects and oxygen-supply following haemodilution with dextran, stroma-free haemoglobin solution and fluorocarbon suspension. Cardiovasc Res 16:194-204.

Paper 8. Elert O, Otterman U. 1979. Cardioplegic hemogobin perfusion: A method of providing optimal myocardial protection. Thorac Cardiovasc Surg 27:245-247.

Paper 9. Rosen AL, Gould S, Lakshman RS, et al. 1979. Cardiac output response to extreme hemodilution with hemoglobin solution of various P_{50} values. Crit Care Med 7:380-383.

Paper 10. Suaudeau J, Fallon JT, Austen WG, Erdmann AJ III, 1978. Stroma-free hemoglobin solution for perfusion of the isolated lamb heart at 38°C. Trans Am Soc Artif Intern Organs, 24:261-269.

Paper 11. Suaudeau J, Fallon JT, Kunica E, Austen WG, Erdmann AJ. 1979. Protection by plasma proteins of the isolated lamb heart perfused with stroma-free hemoglobin at 38°. Ann Surg, 189:322-333.

Paper 12. Feola M, Azar D, Wiener L. 1979. Improved oxygenation of ischemic myocardium by hemodilution with stroma-free hemoglobin solution. Chest 75:369-375.

Paper 13. Moores WY, DeVenuto F, Heydorn WH, et al. 1981. Extending the limits of hemodilution on cardiopulmonary bypass using stroma-free hemoglobin solution. J Thorac Cardiovasc Surg 81:155-162.

Paper 14. Moores WY, DeVenuto F, Heydorn WH, Greenburg AG, Utley Jr. 1982. Effectiveness of stroma-free hemoglobin solution as seen in a right heart bypass swine model. Crit Care Med 10:279-282.

Paper 15. Kim HW, Rowley BA, Feola M, Roberts LA. 1983. Effect of stroma-free hemoglobin on an isolated perfused rabbit interventricular septum. [Abstract No. 2766] Fed Proc 42:765.

Paper 16. Langdale LA, Gould SA, Lakshman RS, et al. 1983. Stroma-free hemoglobin - a myocardial depressant? [Abstract] J Trauma, 23:647.

Paper 17. Dennis RC, Vogel WM, Apstein CS, Valeri CR. 1983. Potent vasoconstrictor substance in stroma-free hemoglobin observed during perfusion of isolated rabbit hearts. [Abstract] In: Advances in Blood Substitute Research, Bolin RB, Geyer RP, Nemo GJ, eds. Prog Clin Biol Res 122:427-428.

Paper 18. Moores WY, White FC, Bloor C, Greenburg Ag, Mark R, Willford DC. 1982. The physiologic effects of oxygen transport by hemoglobin solutions. In: Advances in Blood Substitute Research, Bolin RB, Geyer RP, Nemo GJ, eds. Prog Clin Biol Res 122:89-99.

Paper 19. Moores, WY. 1983. Data Summary. Evaluation of stroma-free hemoglobin solutions as resuscitative fluids for the injured soldier. Number DAMD17-79C-9153.

HEMOGLOBIN SOLUTION EFFECTS ON THE HEART
Review of 19 Research Reports

Paper 1. Riedesel WM II, Erdamar I, Dos SJ. 1973. Functional preservation of canine heart-lung preparation by perfusion with pure haemoglobin solution. Nature 243:530-531.

Human red cells were lysed in hyposmolar phosphate buffer, centrifuged, and filtered. The solution was dialyzed against standard dialyzing fluid adjusted to pH 7.4 for a normal electrolyte balance. The experimental model consisted of canine heart-lung preparations which were maintained in a homeostatic chamber at 37°C. Six dogs were perfused with human hemoglobin solution and six dogs were perfused with heparinized dog blood. Survival time for the hemoglobin-perfused preparations averaged 3.5 hours with the range of 2 to 6 hours, and for the control dogs an average of 8.25 hours ranging from 5 to 12 hours. In both cases, until the end of survival, arterial pressures were comparable and steady. Oxygen saturation ranged from 90-99% in the hemoglobin group but dropped within the 30 to 45% range in the control group. The hemoglobin group tended to become alkalinic with pO_2. Pathologic studies showed no edema and hemorrhage in the hemoglobin infused hearts; which both were present in the control hearts. In addition, subendocardial hemorrhages were larger and occurred more frequently in the blood-perfused hearts. The experiment usually terminated with ventricular fibrillation. Because of this, one can assume that extensive cardiac monitoring occurred and that no arrhythmias were noted in the hemoglobin-infused group up to the time of the death of the heart.

Paper 2. Holdefer WF, Dowling EA. 1974. Experimental use of heterologous stroma-free hemoglobin solution (SFHS) as a whole blood substitute. J Surg Oncol, 6:451-459.

Hemoglobin solution from outdated human whole blood was prepared according to the Rabiner technique. The hemoglobin concentration was 7.6 g% with normal electrolyte concentration in both the hemoglobin solution and the canine whole blood solution, which was used as control. Both contained 15% mannitol. The experimental procedure utilized an in situ canine isolated heart preparation with a dual membrane lung perfusion circuit. For the normal thermic study, two dogs received canine whole blood and two dogs received stroma-free hemoglobin solution. In a separate series of experiments, the effect of hypothermia on this model was studied. The heart was perfused with canine whole blood at 25°C. for 30 min. After this time, the same hearts received stroma-free hemoglobin solution at the same temperature for 30 minutes. Perfusate flows were controlled to maintain a mean arterial pressure of 75 mm Hg. At the end of the study, the hearts were examined by light microscopy for pathologic changes.

The authors determined that the P_{50} was 25 at pH 7.35. Satisfactory cardiopulmonary bypass was maintained for the one hour of perfusion at 37°C with stroma-free hemoglobin solution, but the animal died 45 minutes later in acute pulmonary edema. There was no loss of sinus rhythm during stroma-free hemoglobin perfusion, and following reperfusion with blood in the animals who were studied at the lower temperature, the myocardium remained soft and contracted vigorously. Pathologic studies on the animals who received stroma-free hemoglobin at 37°C and then died showed findings of acute pulmonary edema and hemorrhage and the renal tubule lumen was filled with hemoglobin solution. However, the authors say that none of these morphologic alterations were attributable to stroma-free hemoglobin solution. They attribute the deaths of the animals to hemodilution and hypoxemia.

Paper 3. Yarochkin VS, Koziner VB. 1977. Oxygen supply of the isolated rabbit heart during perfusion with eryhem (hemoglobin solution) and blood. Bull Exp Biol Med 84:1546-1548.

Isolated rabbit hearts were studied. The hearts which were perfused with either a 3% hemoglobin solution or autologous blood diluted with physiologic saline until the hemoglobin concentration was 2.5 to 3 g%. The heart was perfused at a constant pressure of 30 to 35 mm Hg and the perfusion fluid was pumped into the coronary arteries from the oxygenator and collected in the right ventricle where they merge from the coronary sinus and also from the anterior cardiac veins of Thebesius. This experiment was carried out at 38°C. Indices of heart function were determined at 10, 30, and 60 minutes after the beginning of perfusion.

The results show that eryhem was sufficiently saturated with oxygen, and the oxygen concentration, the hemoglobin saturation, and the pO_2 with eryhem corresponded to those with arterial blood. After 60 minutes of perfusion, the pO_2 in eryhem was substantially lower than in blood. Which the authors speculated, was due to the formation of methemoglobin in some degree of denaturation of the protein. The $(A-V)O_2$ difference for eryhem was equal to that for blood, which indicated that oxygen was released to the tissues. In the case of eryhem perfusion and whole blood perfusion, the heart muscle received inadequate oxygen supply, but during perfusion with eryhem the hypoxia was more severe. Both the oxygen concentration and the pO_2 were significantly lower than in the hearts which received blood. There was more marked Acidosis and hypercapnia were more distinguishable in the eryhem group. The oxygen capacity of diluted blood was 1.4 volume % higher than for Eryhem, even though the hemoglobin concentration was only 0.3 g% higher. This corresponded to eryhem having only 72% of the oxygen capacity of hemoglobin. They also noted that the eryhem dissociation curve was shifted slightly to the left which interfered with oxygen release to the tissues. The hearts receiving eryhem exhibited both vasodilation, as manifested by an increase in coronary blood flow, and a greater oxygen utilization. The coronary blood flow was 50% greater during perfusion with eryhem than with perfusion with blood. The relative bradycardia observed in the experiment with eryhem did not prevent the increase in coronary blood flow.

Paper 4. Labrude P, Duvelleroy M, Vigneron C. 1977. [The behavior of haemoglobin solution in experimental circuits. Possible utilization of the results on potential use in vivo.] Title translated from the French. English Summary. Agressologie, 18:21-27.

In previous studies the authors showed that hemoglobin solution perfused through an isolated closed circuit rat heart perfusion model resulted in cardiac arrest secondary to coronary occlusion. This occlusion was due to clogging by breakdown products of the hemoglobin solution. The current paper discusses experiments involving mixing of the hemoglobin solution and its circulation in vitro. The hemoglobin was unstable in contact with the perfusion circuit. Macromolecules, in particular albumin, delayed the breakdown of the solution. The evidence suggested that the instability of free hemoglobin in solution was a major obstacle to its use.

Paper 5. Biro GP, Beresford-Kroeger D, Smith BV. 1978. Myocardial oxygen-supply during hemodilution with stroma-free hemoglobin and methemoglobin solutions. In: Blood Substitutes and Plasma Expanders. [New York: Alan R. Liss, Inc.] 213-222.

In this series of experiments, anesthesized dogs underwent rapid isovolemic hemodilution with 6% stroma-free hemoglobin solution or with 6% methemoglobin solution. This resulted in a control group which was identical to the experimental group except for the ability to transport oxygen. The hemoglobin solution was prepared and supplied by Warner-Lambert Research Institute of Morris Plains, NJ. Although Biro et al do not reference a protocol, presumably the hemoglobin solution was the same material Savitsky used and documented a Warner-Lambert Research Institute in the late 1970s (Clin Pharmacol Therap 23:73-80, 1978). Washed red cells were lysed in hypotonic phosphate solution and filtered twice through 0.2 micron Millipore filters. The solution was dialyzed against sodium bicarbonate solution until the electrolytes were similar to human plasma.

Dogs were anesthesized with Penthrane/N_2O/O_2 and were ventilated. A thoracotomy was performed on each animal and the coronary arteries were isolated. Rapid isovolemic exchange-transfusion was performed and approximately 50% of the estimated blood volume was removed. In 9 dogs this was replaced by 6% hemoglobin solution and in 8 dogs by 6% methemoglobin solution.

Isovolemic hemodilution was followed by equal reduction in the hematocrit and total hemoglobin concentration in both groups. In the hemoglobin exchange group, mean O_2-capacity in the plasma phase was 4.7 mL/dL, in contrast to 0.5 mL/dL, in the methemoglobin exchange group. The arterial O_2 content was reduced in both groups, to [illegible] mL/dL in the hemoglobin exchange group and to 8 mL/dL in the control group. The difference between the two is due to the presence of "normal" hemoglobin (3 g/dL) in the plasma phase in the former. Following hemodilution, the hemoglobin-exchanged experimental group exhibited a reduction in the coronary sinus O_2 content from 5 to [illegible] mL/dL. In this group, oxygen extracted by the heart fell from approximately 75 to 66%, in association with a drastic decrease in pO_2 in coronary sinus blood, to as low as 7 mm Hg. In contrast, the methemoglobin exchanged group showed a marked reduction in coronary [illegible] content from [illegible] to [illegible] mL/dL and oxygen extraction by the heart [illegible] approximately [illegible] to 75%, in association with a modest rise [illegible] coronary sinus pO_2. An early significant bradycardia was seen in the hemoglobin exchanged group but not in the methemoglobin-exchanged group. In spite of the reduced hematocrit, both groups failed to exhibit a significantly augmented cardiac output. There is no difference between groups in the maximum rate of change of ventricular pressure with time, while stroke work of the left ventricle increased

significantly in the hemoglobin-exchanged group. Myocardial blood flow showed marked and statistically significant increments in both groups. The increments were greater in the methemoglobin-exchanged group than in the hemoglobin-exchanged group. Myocardial oxygen delivery was maintained at near-control values in both groups. There was 4 mL/dL extra oxygen in the plasma phase of the hemoglobin-exchanged group which allowed maintenance of normal oxygen delivery with a smaller flow increment than that required in the methemoglobin exchanged group which did not have the extra oxygen. In neither group was the oxygen-supply significantly compromised. There was redistribution of blood flow to the different layers of the left ventricular wall in the two groups. In the hemoglobin-exchanged group, the endocardial layer was favored for redistribution of blood flow. In the methemoglobin-exchanged group, the epicardial layer was preferentially perfused. Because of this, the estimated oxygen delivery to the endocardium appeared better in the hemoglobin-exchanged group. The authors note that the coronary sinus pO_2 markedly reduced in the hemoglobin-diluted dogs, but that there was an observed elevation of coronary sinus oxygen saturation in these dogs. They attribute this to the presence of extra-erythrocytic hemoglobin, which was responsible for shift in the composite oxyhemoglobin dissociation curve following hemodilution with stroma-free hemoglobin solution by approximately 9 mm mercury at 50% saturation. They estimated that at the pO_2 prevailing in coronary venous blood almost all the oxygen unloaded came from the intra-erythrocytic hemoglobin, while the plasma phase hemoglobin still bound substantial amounts of oxygen. They felt that the increased affinity of the extra-erythrocytic (unmodified) hemoglobin caused a substantial reduction in the mean myocardial pO_2, but that it was possible that perfusion became more homogeneous, and myocardial hypoxia of a magnitude sufficient to impair contractile function was prevented.

Paper 6. Biro GP, Beresford-Kroeger D. 1980. The effect of hemodilution with stroma-free hemoglobin and dextran on collateral perfusion of ischemic myocardium in the dog. Am Heart J 99:64-75.

This paper is in many ways similar to the paper by Feola in Chest, 75:369-375 (1979). The blood used in the paper by Biro and Beresford-Kroeger is stroma-free hemoglobin solution prepared from packed human erythrocytes by the method of Rabiner et al (1967). This material was tested in rapid exchange transfusion in rabbits and dogs. It was free of pyrogens and had only an occasional, transient, less than 2 min hypotensive reaction. Some slowing of heart rate was noted. The material was 8.1 g%, had 5% methemoglobin, sodium was 122, potassium 2.5, chloride 95, pH 7.17, osmolarity 278, O_2 capacity 10.2 mL/100cc. The solution was sterile on culture. The protocol consisted of anesthetizing dogs with Nembutal. The left descending coronary artery was isolated. It was occluded after baseline physiologic measurements were made. Occlusion of the left anterior descending artery was followed by transient dysrhythmias in only 4 dogs; 3 of which subsided spontaneously within 5 minutes. The one dog which had persistent dysrhythmias was discarded. No drugs, other than supplemental anesthetic, were given, i.e. no anti-arrhythmic drugs. One hour after occlusion a rapid-exchange transfusion was performed. Approximately 30 to 35 mL/kg of blood was removed from the femoral artery and an equivalent volume was reinfused in the femoral vein. Nine dogs received stroma-free hemoglobin solution (Group 1), 9 dogs received their shed blood (Group 2) and 6 dogs received a 6% solution of Dextran-70 (Group 3). Physiologic measurements were then made for the subsequent 2 hours. Myocardial blood flow and distribution were estimated by the trapping of radionucleide labeled microspheres. As a result of the occlusion, before infusion, heart rate remained constant, mean arterial pressure tended to rise, cardiac output and stroke volume tended to fall, while left ventricular end-diastolic pressure rose. None of these were statistically significant between groups. There was a consistent decrease in the left ventricular dP/dT. All groups exhibited a fall in coronary sinus pO_2 and oxygen saturation. Blood flow to the normal myocardium at 1 hour after occlusion was elevated, whereas blood flow to the ischemic zone was reduced. After infusion, the transmural flow distribution was significantly reduced in the SFHS group only.

The hematocrit was reduced in groups 1 and 3 as would be expected from hemodilution. In Group 1 with the stroma-free hemoglobin solution, the O_2 capacity was 5 mL/dL; in Group 3, the Dextran group, it was 9.6 mL/dL; in Group 1, the O_2 content was 4 mL/dL but this was still 5 mL/dL lower than in Group 2. Heart rate and blood pressure post-exchange transfusion showed some differences, but the investigators did not mention whether these differences were significant or not. The heart rate in the stroma-free hemoglobin

exchange group dropped compared to the other groups and remained below the heart rates of the groups which received blood or Dextran-70 exchanges. No arrhythmias were noted by EKG monitor. The mean arterial pressure of the groups which received auto-transfusion was the same as the mean arterial pressure for the group which received stroma-free hemoglobin solution for the entire post-transfusion period. It was lower in both Groups 1 and 2 than in groups which received hemodilution with Dextran-70. Only Group 3 (Dextran-70) exhibited elevated cardiac output when compared to the other groups. Group 1 (SFHS) exhibited a significant rise in dP/dT as compared to the post-occlusion depressed levels, but only at two of the measurement periods. Two minor and not statistically significant differences were also evident: Group 1 showed the most marked fall in arterial blood pressure and lowest left ventricular end diastolic pressure (LVEDP). In terms of coronary sinus blood, there was a rise of approximately equal magnitude in pO_2, O_2 saturation and all 3 groups returned to the pre-occlusion range. Blood flow to the normal myocardium rose only marginally in Group 2, but increased significantly in both hemodilution groups. In contrast in the ischemic and marginal zones, although there were significant increments in blood flow, O_2 delivery was restricted in all 3 groups with two notable exceptions: In Group 1, endocardial O_2 delivery after hemodilution with SFHS was higher in the ischemic zone and it was significantly higher in the marginal zone. When compared to the pre-hemodilution level in the same animals as well as when compared to the post-exchange transfusion values in the other groups, the data indicate a significant improvement in endocardial perfusion following hemodilution with SFHS in the normal as well as in the ischemic myocardium. One hour after the exchange transfusion, the "washout" of CPK from the heart increased significantly in Groups 2 and 3 but was reduced in Group 1. By weighing the three zones of myocardium, the ischemic and marginal zones were somewhat smaller in Group 1. The authors noted that this difference may have indicated an apparent underestimation of these zones because of a difficulty in visual demarcation in the animals hemodiluted with SFHS. They commented that this underestimation was reflected in the somewhat lower blood flow, expecially in the endocardial layer, found in the ischemic zone in this group, at the 1 hour post-occlusion period, which indicated that this zone may have been underestimated in comparison with the other groups. The authors suggested that the experiment revealed three lines of circumstantial evidence pointing to an improved collateral perfusion of the marginally ischemic myocardium, particularly to the subendocardial layers, following hemodilution with SFHS. First, Group 1 (hemoglobin) exhibited the greatest increment in subendocardial blood flow and in O_2 delivery. Secondly, in contrast to the increasing output of CPK in Groups 2 and 3, there was a reversal of this trend in Group 1. Presumably, this indicates a reduction in the mass of myocardium leaking the enzyme. Thirdly, although

statistically not consistently significant, Group 1 exhibited the best left ventricular performance, returning to the pre-occlusion level. There is a further possibility of a passive mechanical factor contributing to the improved collateral perfusion. Flow in a maximally dilated vessel, as these collaterals presumably were, is also proportional to the left ventricular end-diastolic pressure, because of the vascular "waterfall" phenomenon. As a result of the lower end-diastolic pressure in Group 1, there is less compression than in the other groups, allowing better perfusion to the maximally dilated collaterals. The fact that a similar phenomenon is not seen in the Dextran-hemodiluted group suggests that a factor other than reduced impedance to ejection is also involved. Blood values measured in the coronary sinus revealed no significant differences between the groups. The pO_2 and oxy-hemoglobin saturation in coronary sinus blood reflects the integrated effects of a spectrum of oxygen extraction by various regions of the myocardium.

In summary, they noted that following the exchange transfusions, blood flow to the ischemic zone increased in all groups, but only marginally in Group 2. The greatest increment was seen in the SFHS hemodiluted group in which endocardial flow increased by 83% and epicardial flow increased by 45%. These resulted in the greatest improvements in oxygen delivery. Significant increases in blood flow were seen in Group 3 as well, but oxygen delivery was less adequate. Group 1 also exhibited the lowest output of CPK from the heart and was the only one in which the indices of left ventricular performance (dP/dT and EDP) were returned to the pre-occlusion level. These findings suggest the possibility that reduction of blood viscosity by dilution with SFHS improves collateral perfusion of the ischemic myocardium.

Paper 7. Biro G. 1982. Comparison of acute cardiovascular effects and oxygen-supply following hemodilution with dextran, stroma-free haemoglobin solution and fluorocarbon suspension. Cardiovasc Res, 16:194-204.

Biro selected as the study model an in situ right heart bypass with dogs. These animals were anesthetised with sodium pentobarbitone. They were intubated and a right heart bypass was performed. A rapid exchange transfusion was performed in which approximately 40% of the animal's estimated blood volume was removed via the femoral artery and replaced with one of three solutions by the femoral vein. In Group 1, the blood volume was replaced in 9 dogs with 8% stroma-free hemoglobin solution prepared from outdated packed human erythrocytes by the method of Rabiner et al (J Exp Med 126:1127-1142, 1967). In Group 2, 9 dogs received Fluosol-DA 20%. In Group 3, 8 dogs received dextran-70 (6%). Group 4 consisted of 8 dogs which received their own blood for reinfusion.

The hematocrit was as a result reduced from 18 to 22% by this isovolemic hemodilution. Comparable hemodilution and comparable reduction in whole blood viscosity was not followed by similar changes in cardiac output; dextran and Fluosol-diluted groups of dogs showed significantly elevated cardiac output, while hemoglobin-diluted groups did not show elevated cardiac output. As a result, systemic O_2 transport was better maintained with dextran and Fluosol. Myocardial blood flow increased in all three hemodiluted groups, but oxygen supply was not similar. The hemoglobin-diluted group showed inadequate O_2 supply, suggested by a fall in coronary sinus pO_2; dextran diluted group exhibited adequate O_2 supply suggested by maintaining coronary sinus pO_2, while the Fluosol-diluted group enjoyed excessive O_2 supply indicated by a markedly elevated pO_2 in coronary sinus blood. There was little difference in heart rate and arterial blood pressure in the 4 groups except for a transient bradycardia in the SFHS diluted dogs of Group 1. Cardiac output was maintained at control level in all four groups. However, in these groups, Groups 2 (Fluosol) and 3 (Dextran) had an immediate and significant elevated cardiac output, but the elevation was less sustained and the Fluosol-diluted animals than in the dextran diluted group. The estimated systemic O_2 transport rate, which was the product of cardiac output and arterial O_2 content, was lowest in Group 1, the SFHS group, because of the failure of the cardiac output to rise; it was almost up to the control level in Group 2 initially, falling subsequently to the level of the dextran diluted group. The indices of left ventricular performance indicate best sustained left ventricular dP/dt in Group 3 (dextran-70) accompanied by marginal elevations in LVEDP. There was no significant difference between the other three groups in this respect. The calculated systemic and coronary vascular resistances at 1-hour after exchange showed that

there was a marginal rise, although not statistically significant, in systemic vascular resistance in Groups 1 and 2, in contrast to a significant fall in Group 3. Estimated total vascular hindrance tended to rise in all three groups. In contrast, coronary vascular resistance fell markedly in all three groups. There are obvious differences between the groups in the oxygen available to and extracted by systemic circulation. Each of the hemodynamic groups exhibited a reduction in oxygen content, as well as a rise in pO_2, in the arterial blood. The former is due to the dilution of the erythrocytic hemoglobin concentration of whole blood; this cannot be balanced by a significant O_2-transport capacity in the plasma phase, since the erythrocytic O_2 capacity is still 5 to 6 times greater than in the plasma phase. The rise in arterial pO_2 is presumably due to a more even topographic distribution of the V_A/Q ratios in the lung, since ventilation and inspired pO_2 were unaltered. There was a surprising similarity in the mixed venous O_2 content in the three hemodiluted groups, in the face of large differences in pO_2. While dextran dilution was followed by only a small fall in mixed venous pO_2, SFHS dilution was followed by a significantly greater fall, while a marked rise occurred in the Fluosol diluted animals. The authors postulated that the fall in the SFHS diluted group was related to the high O_2 affinity of SFHS which rendered the tissues' access to the extra-erythrocytic oxygen more difficult. In contrast, the linear O_2 loading/unloading characteristics of Fluosol appear to present an apparent advantage, provided that high alveolar pO_2 can be maintained, thus allowing the extraction of substantial amounts of O_2 at a higher venous pO_2. The oxygen available to and extracted by myocardium showed that in the dextran diluted group the flow increment was of such magnitude that coronary sinus pO_2 was only marginally reduced. In sharp contrast, the extra-erythrocytic O_2 carried by SFHS was not available to the tissue and the flow-increment was not adequate, which necessitated a marked enroachment on venous reserve. The Fluosol-diluted group exhibited the opposite phenomenon; in these, adequate oxygen extraction was achieved at a significantly higher pO_2 in the coronary sinus, thus appearing to be at a relative advantage. This view of relative advantage is predicated on the assumption that changes in coronary sinus pO_2 parallel, at least in a semi-quantitative sense, those prevailing at the tissue level in the myocardium. This assumption, in the absence of direct measurements of myocardial pO_2, was not substantiated by data in this paper, but it is conceivable that somewhat better myocardial O_2 supply in the Fluosol-diluted group may be responsible for their marginally superior ventricular performance in this study.

The currently excepted view of coronary sinus blood flow regulation suggests that regulating coronary arteriolar resistance to keep flow adequate to maintain relatively constant myocardial pO_2 is dependent on adenosine as a mediator regulating arteriolar tone.

Perhaps under these experimental conditions there was a failure of this autoregulatory feed-back mechanism. This suggests that the failure of adequate coronary vasodilatation in the SFHS diluted dogs was related to interference with the normal movement and/or metabolism of the vasodilating mediator. This appeared more plausible, since normal erythrocytes are capable of rapid degradation of adenosine. This adenosine deaminase activity is not tightly bound to the erythrocyte membrane and was perhaps not removed completely during the procedure to remove stroma fragments. Adenosine deaminase activity in the SFHS solution was significantly lower than in unpurified hemolysate of dog blood. The author concluded in this paper that similar degrees of hemodilution may not be followed by comparable changes in hemodynamics and O_2 supply, because of the different O_2 unloading characteristics of SFHS vs Fluosol. In this regard Fluosol was found superior to SFHS.

Paper 8. Elert O, Otterman U. 1979. Cardioplegic hemoglobin perfusion: A method of providing optimal myocardial protection. Thorac Cardiovasc Surg, 27:245-247.

The protocol consisted of a 1-hour period of normothermic cardiac arrest in dogs. This was produced by infusion of room temperature hemoglobin solution which contained all of the following cardioplegic agents: Mg-aspartate, K-aspartate, and procaine. After this 1-hour cardioplegia, all the hearts reversed promptly, and after a 10-15 minute recovery phase, the hearts showed normal pressures and output. Metabolic investigations of left ventricular myocardial tissues showed a fall in adenosine triphosphate of up to 46% after 60 minutes, and a 63% fall in phosphocreatine as compared to the initial levels. Resumption of perfusion after 30 minutes ischemia led to a more rapid increase in the low ATP and PKr levels during the reperfusion phase.

Comparative studies of myocardial metabolism showed that this level of energy-rich phosphates and lactic acid was achieved at 15 minutes of pure normothermic ischemia. It was also shown that cardioplegia with Mg-aspartate-procaine and mild hypothermia offer similiar myocardial protection for only 40 to 45 minutes as compared with 1-hour protection from normothermic hemoglobin cardioplegia.

The authors concluded that a 6.4% stroma-free hemoglobin solution containing cardioplegic additives showed improved myocardial protection during cardiac arrest. This solution produced optimal and easily reversible cardiac arrest, together with optimal oxygen transport and oxygen yield in the myocardium, similar to that provided by whole blood.

Paper 9. Rosen AL, Gould S, Larshman RS, et al. 1979. Cardiac output response to extreme hemodilution with hemoglobin solutions of various P_{50} values. Crit Care Med, 7:380-383.

The hemoglobin solution the investigators used was essentially as described by Sehgal et al. Ear Surg Res 11 (suppl.2):43, 1979. It was pyridoxalated but not polymerized. The concentration was between 5.7 and 8.0 g/dl. The P_{50} was from 12 to 59 mmHg, osmolarity was 290 to 310 mOsm/kg and the colloid osmotic pressure varied between 20 and 25 mmHg. Adult baboons were tranquilized, anesthetized, and intubated. They recieved the following medications: Ketamine, atropine, thiopental, and D-turbocurarine. These animals underwent right heart catheterization and also had continuous electrocardiographic monitoring. The hemoglobin solutions were prepared with various P_{50}s by varying the amount of pyridoxal phosphate coupling to the hemoglobin. Baboons were randomized to receive the hemoglobin solutions. A baseline set of measurements was obtained and then exchanged transfused to a hematocrit below 6%, and again hemodynamic measurements were taken.

The starting hematocrit of 35% fell to 1.5%; the functional whole blood hemoglobin concentration went from 11.6 to 4.4 g/dl; and the whole blood in vivo P_{50} fell from 31 to 18 mmHg. These changes were significant. The cardiac output fell from 3.2 to 2.5 liters/min but was not significant. The heart rate increased from 108 to 141 beats/min, but the stroke volume, mean arterial pressure, and mixed venous PO_2 all decreased significantly. There were no changes in pulmonary capillary wedge pressures, intravenous pressure, mean pulmonary artery pressure, systemic vascular resistance, oxygen consumption, and oxygen content difference. There was no correlation between the cardiac output and the whole blood in vivo P_{50}, nor was there a correlation between the P_{50} and the heart rate or stroke volume.

The authors commented that in previous work with babcons, an increase in cardiac output had been noted in normal volume anemia caused by exchange transfusion with Dextran-75, but not after total exchange transfusion with stroma-free hemoglobin. In the total exchange, case the hemoglobin concentration did not fall below 6 g/dl and the P_{50} was 14 mmHg. The hypothesis of the paper is that the cardiac output did not increase with decreasing hemoglobin concentration because the hemoglobin concentration might have been just above the critical value or that a large fall in P_{50} led to myocardial hypoxia. They have now demonstrated that the hemoglobin concentration, per se, does not appear to be the critical stimulus for an increase in cardiac output with hemoglobin solution. In addition, the position of the hemoglobin-oxygen dissociation curve did not appear to influence the hemodynamic responses. They concluded that

the physiologic response to anemia in the presence of hemoglobin solution appears different than that observed in the absence of plasma O_2 carriers.

Paper 10. Suaudeau J, Fallon JT, Austin WG, Erdmann AJ III. 1978. Stroma-free hemoglobin solution for perfusion of the isolated lamb heart at 38° C. Trans Am Soc Artif Intern Organs, 24:261-269

Hemoglobin solution was prepared from washed sheep red blood cells. The red cells were lysed in hyposmolar phosphate buffer and underwent sequential filtration and ultrafiltration. Molecular sieve filters were used so that molecules less than 5000 daltons were retained. Hemoglobin solutions varied from 4.4 to 11%. Methemoglobin was 0.3 g/dl; P_{50} was 32; electrolytes were physiologic; osmolality was 300; pH 7.5. The procedure consisted of isolating lamb hearts and connecting them to a perfusion circuit, consisting of a pump, a lung membrane, and dialysis system to maintain nutrition. There was a continual, gradual turnover of hemoglobin. Perfusion occurred at 38° C.

In lamb hearts which were perfused with whole fresh lamb blood, left arterial perfusion was supported by the heart for 9 to 25 hours and excellent performance occurred throughout. Left ventricular function curves were normal. There was no change in coronary vascular resistance, myocardial oxygen consumption, carbon dioxide release, or arterial venous pH differences. Some hearts developed minor degrees of subepicardial and subendocardial hemorrhage; none became edematous. Microscopically there was good preservation of myocytes. In the group which received perfusion with 7% stroma-free hemoglobin solution, left ventricular function was abnormal. Left atrial perfusion occurred up to 4 hours. Coronary vascular resistance was lower than control hearts and coronary flow was higher. Perfusate pH, blood gases, and myocardial oxygen consumption were the same as control and did not change during left atrial perfusion, even with the onset of ventricular failure. These hearts showed diffuse hemoglobin staining of the myocardium soon after the onset of perfusion and later they became edematous. Microscopically there was marked cellular and interstitial edema; myocytes were intact except for the mitochondria which were at times swollen with loss of cristie and matrix granules. Significant hemoglobin accumulation was found in the interstitium and areas of coagulation necrosis were present. In hearts that received 11% stroma-free hemoglobin, 2 of the hearts performed better than hearts that received 7% hemoglobin; however, 3 others did not tolerate left atrial perfusion. Coronary vascular resistance, perfusion flow, perfusate pH, and arterial gases were the same as 7% hemoglobin, but the 11% hemoglobin hearts consumed more oxygen and developed early hemoglobin staining of the myocardium and prominent edema. Histologically they were similar to the group receiving 7% hemoglobin, but the changes were more severe and diffuse. Another group received 8% hemoglobin with 7% albumin. These hearts were able to generate less ventricular stroke work than for 7% hemoglobin alone. They failed after only 1 1/2 hours of left atrial perfusion. Coronary

vascular resistance was high and perfusate flow correspondingly low. The AV pO_2 and pH differences were higher than these values for the other groups. Despite low perfusion flow, these hearts extracted as much oxygen as control hearts, and the blood gases did not suggest tissue hypoxia or acidosis. The hearts appeared normal after 8 hours of perfusion. Microscopically there was less edema than the other 2 groups and extravasated hemoglobin was less conspicuous. Another group received 4.4% hemoglobin with 5.5% albumin. They did not maintain ventricular work with left atrial perfusion for longer than 1 1/2 hours, but during that time they performed better than the hearts which received 7% hemoglobin. Coronary vascular resistance, coronary flow, oxygen consumption, perfusate pH, and arterial gases were the same as the values for the group which received 7% hemoglobin. Histologically they appeared similar to the group which received 8% hemoglobin with 7% albumin, but they had more weight gain. In general, the hearts were able to be maintained with hemoglobin solution for up to 5 hours. However, as noted above, there were many problems. Increasing the oxygen-carrying capacity of their perfusate by increasing the hemoglobin from 7% to 11% did improve ventricular performance in 2 of 5 hearts, but also led to more extensive morphologic damage. Increasing the colloid osmotic pressure with a perfusate by adding albumin markedly improved the morphologic preservation but the viscosity of the perfusate was so high that coronary flow was reduced by 50% and AV pH was doubled. These hearts could not perform any significant work. The last solution, the 4.4% hemoglobin with 5.5% albumin, had a lower viscosity than the 8% hemoglobin with 7% albumin and again there was good morphology, yet coronary blood flow and ventricular performance were the same as for the 7% hemoglobin alone group. None of the hemoglobin preparations was able to equal the performance of myocardium with whole lamb blood. Hemoglobin perfused hearts consumed as much or even more oxygen than hearts perfused with whole blood but could produce only 1/2 or 1/3 the cardiac work.

Paper 11. Suaudeau J, Fallon JT, Kunica E, Austen WG, Erdmann AJ. 1979. Protection by plasma proteins of the isolated lamb heart perfused with stroma-free hemoglobin at 38°C. Ann Surg 189:322-333.

Stroma-free hemoglobin solution was prepared from washed sheep red cells which were lysed in hyposmolar phosphate buffer. This solution was mixed with saline to adjust the electrolyte and buffer concentrations and was ultrafiltered to remove molecules other than the size of hemoglobin. The final material was between 4.5 and 11 g% hemoglobin, 0.3 g% methemoglobin; the P_{50} was 32; osmolarity, 300; pH 7.5 at 38°C. Hearts were removed from 2 to 3 month-old lambs and placed in a preservation chamber which was attached to a venous reservoir, a central reservoir, a Millipore filter, membrane lung exchanger, and a dialysis system. For the hearts which received stroma-free hemoglobin solution, a small amount was removed from the circuit constantly to minimize methemoglobin accumulation. The stroma-free hemoglobin was also dialyzed against blood from a donor sheep across an artificial kidney to prevent nutrient depletion and metabolic waste product accumulation during the perfusion. For the hearts which received whole blood, exchange against whole blood did not occur but whole blood was replaced with fresh whole blood constantly during the experiment. There were 5 groups. One group received whole fresh lamb blood; the second group received stroma-free hemoglobin, 7% (Group A); Group B received 11% stroma-free hemoglobin; Group C received 8% stroma-free hemoglobin with 7% bovine albumin and 1 part microfilter plasma in order to increase the colloid osmotic pressure; Group D received a compromised solution containing less albumin and less hemoglobin in order to balance the colloid osmotic pressure and viscosity. In general, they were able to perfuse isolated lamb hearts with 7% stroma-free hemoglobin at 38°C. for 8 hours, by using left atrial perfusion for the first 2 hours followed by aortic perfusion. They found sustained contractions and no rise in coronary vascular resistance, normal arterial venous pO_2, pCO_2 and pH differences, and normal myocardial oxygen consumption. Stroma-free hemoglobin perfused hearts did not perform as well as control hearts perfused with blood. They were unable to sustain prolonged left atrial perfusion and developed extravasation of hemoglobin and interstitial edema as well as myocytic and vascular-endothelial cell damage. For Group A (7% SFHS), the performance was inferior to that of control hearts. None could tolerate left atrial perfusion (LAP) for more than 2 hours, whereas control hearts had at least 8 hours of LAP. They had lower ventricular function, lower mean coronary resistance and higher coronary flow than control hearts. In contrast to control experiments, oxygen consumption, carbon dioxide production and arterial venous pH differences increased to a peak during left atrial perfusion and then decreased during aortic perfusion. This was found in all animals receiving stroma-free hemoglobin solution. After the beginning of perfusion, diffuse hemoglobin staining of the

myocardium developed and the heart appeared edematous. There was an increase in myocardial wall thickness and in heart weight. Microscopically there was prominent interstitial edema with intracellular edema and extravasation of hemoglobin. Subendocardial and midmural areas of coagulation necrosis were present in all but two of these hearts. When the hemoglobin concentration was raised to 11% (Group B) as an attempt to increase oxygen-carrying capacity, this did not improve heart function. Three of the hearts from this group could not sustain left atrial perfusion at all. Ventricular performance with aortic perfusion was the same as for Group A. Coronary resistance rose during the last hours of perfusion and mean oxygen consumption was higher than for Group A. Morphologic changes were more severe and more diffuse than for Group A. In addition, there were ultrastructural changes seen in endothelial and myocardial cells consisting of swelling and prominent mitochondrial changes. When 7% albumin was added to 8% hemoglobin (Group C), myocardial performance with left atrial perfusion was similar to Group A. Myocardial contractility was not depressed following the left atrial perfusion trial in contrast to Group A. Mean coronary resistance was higher and mean coronary flow lower than for Group A perhaps due to a higher viscosity of the blood. Coronary vascular resistance did not change during the perfusion. Mean oxygen concentration was slightly higher than for Group A. Grossly, the hearts appeared better preserved than for Group A. Microscopically there was markedly less edema than in Groups A or B, and extravasated hemoglobin was less conspicuous. However, eosinophilic plugs were present in many small vessels and coagulation necrosis was evident throughout the subendocardium. Electron microscopy showed minimal intracellular edema and only occasional cells with degenerating mitochondria. For Group D with the lower albumin of 5.5% and hemoglobin of 4.4%, these hearts did not sustain left ventricular work with left atrial perfusion for longer than 1 1/2 hours, but their ventricular performance was still slightly better than Group A hearts during the same period. Although the viscosity of the perfusate was similar to Group A, coronary vascular resistance was lower than in the other groups, although there was no significant change in coronary resistance. Grossly the hearts appeared as well preserved as for Group C, but had a greater weight gain. Histologically and ultrastructurally they were similar to Group C.

In summary, isolated lamb hearts were perfused for 8 hours at 28°C. with stroma-free hemoglobin solution. The preservation of cardiac structure and function was studied. Control hearts perfused with blood (N=5) developed no ventricular failure or significant weight gain (12%±5), showed no alteration of cellular ultrastructure, and little interstitial edema. Hearts perfused with 7% (N=7) or 11% (N=5) stroma free hemoglobin solution contracted less well, became [illegible] weight gain, respectively) and showed altered

mitochondria, capillary endothelial swelling and hemoglobin extravasation into the interstitial space. The addition of 5 to 7% albumin to stroma-free hemoglobin solution (N=9) markedly reduced interstitial edema (weight gain 11%), preserved mitochondria, prevented endothelial swelling, and limited transcapillary escape of hemoglobin. Thus isolated hearts perfused with stroma-free hemoglobin solution develop vascular endothelial damage and an increase in capillary permeability. The addition of plasma proteins to the perfusate protects against this injury.

Paper 12. Feola M, Azar D, Wiener L. 1979. Improved oxygenation of ischemic myocardium by hemodilution with stroma-free hemoglobin solution. Chest 75:369-375.

Dogs were anesthetized and the left anterior descending coronary artery was occluded for 3 hours. The oxygenation of the myocardial tissue was monitored by a polarographic technique capable of recording simultaneously the oxygen tension, the pO_2 of myocardial tissue and electrograms. Ischemic injury was monitored by means of ST segment elevations on myocardial and epicardial electrograms. The volume of myocardial infarct was measured at the end of each experiment by incubation of transverse slices of left ventricle in a solution of nitroblue tetrazolium and by separation of the unstained (ischemic) from the stained (normal) portions. The dogs were prepared for this experiment by anesthesia with sodium pentobarbital; they were intubated and ventilated on a respirator; the chest was opened and the electrical monitors were placed on the heart and the pressure monitors were inserted into various chambers of the heart. There was a note in the procedure that to reduce the occurrence of ventricular fibrilation lidocaine hydrochloride was infused at the rate of 2 mg/min for the duration of the experiment. This might explain why there is no mention of arrhythmias in the dogs receiving hemoglobin. Of course, with all the different epicardial electrodes and tubes placed on and in the heart, certainly some degree of ventricular irritation would occur regardless of the presence of hemoglobin solution. In one group of dogs, hemodilution was performed after 15 minutes of ischemia by exchanging blood with a stroma-free hemoglobin solution from a hematocrit of 45 to a hematocrit of 23. Changes occurring in this group were compared to those occurring in dogs that did not undergo hemodilution, underwent hemodilution with Dextran-75, or were retransfused with whole blood. Hemodilution with hemoglobin reduced aortic and left ventricular filling pressures while increasing coronary blood flow, increased myocardial pO_2, lowered ST segment elevation of both myocardial and epicardial electrograms, and reduced the volume of myocardial infarct. These effects were unmatched by hemodilution with dextran or infusion with whole blood. In dogs with hemoglobin solution, the mean left atrial pressure was lowered, the aortic pressure was maintained, the cardiac index was improved over controls, coronary blood flow through the left anterior coronary artery was improved, the myocardial pO_2 was improved over controls and the mean ST segment elevation for epicardial and myocardial tissue was decreased significantly. They felt that hemodilution tended to increase the myocardial supply of oxygen while reducing the demand for oxygen. Hemodilution with hemoglobin decreased the volume of infarct in myocardium by 58% compared to the untreated group, while transfusion with whole blood increased the volume by 44%.

Paper 13. Moores WY, DeVenuto F, Heydorn WH, et al. 1981. Extending the limits of hemodilution on cardiopulmonary bypass using stroma-free hemoglobin solution. J Thorac Cardiovasc Surg, 81:155-162.

Hemoglobin solution was prepared by the technique of DeVenuto, et al, 1977. Hemoglobin concentration was 7 g%; methemoglobin 3.5%; electrolytes were physiologic; pH 7.35; P_{50} 14.5. Small pigs were given halothane for induction of anesthesia, nitrous oxide for maintenance and succinylcholine for paralysis. A tracheostomy was placed and the animals were intubated. They were connected to an extracorporeal perfusion circuit. The pigs were initially hemodiluted to a hemoglobin of 10 g% with their temperature kept at 37°. Their rate was maintained with ventricular pacing at 140 beats/min after blocking the conduction system with injection of formalin. Preload and afterload were controlled and all of the appropriate myocardial measurements taken. Animals were exchange transfused with stroma-free hemoglobin solution or with 7% albumin solution to a hematocrit of 5%. At this point in the in situ right heart bypass model, preload, afterload, and heart rate were all controlled. The results were as follows: Myocardial performance following albumin solution exchange could not be sustained on right heart bypass and these animals had a stroke volume of 0 at a left ventricular and diastolic pressure of 14 torr. Stroma-free hemoglobin solution animals had a significant drop in stroke volume at 14 torr following exchange, but this 50% performance level could be sustained. Coronary blood flow rose and myocardial oxygen consumption fell in both groups, although the statistically nonsignificant mean differences were less with stroma-free hemoglobin solution. Arterial-coronary sinus oxygen differences fell significantly with albumin solution and non-significantly with stroma-free hemoglobin solution. Lactate production occurred in both groups but was greater with the albumin than with stroma-free hemoglobin solution. No changes in myocardial tissue gases were noted in either group. Although myocardial performance decreased and some lactate production occurred with stroma-free hemoglobin solution, they concluded that with these comparative results there was promise in the eventual utilization of an oxygen carrying agent such as stroma-free hemoglobin solution to extend the limits of hemodilution to a hematocrit value of 5% or less. With all the different drugs on board plus the ventricular pacing these hearts were receiving, of course it would have been difficult to observe any arrhythmogenic effect of the hemoglobin solution. No pathology study was done on these hearts.

Paper 14. Moores WY, DeVenuto F, Heydorn WH, Greenburg AG, Utley JR. 1982. Effectiveness of stroma-free hemoglobin solution as seen in a right heart bypass swine model. Crit Care Med 10:279-282.

This is essentially the same paper with the same data as Moores et al, J Thorac Cardiovasc Surg, 1981. The only difference is that a third group was added in which the hematocrit was decreased to 10% with 7% bovine albumin solution in addition to a group which is already reported in the last paper where the hematocrit was decreased to 5% using 7% bovine albumin solution. There conclusions were essentially as reported in the last paper and are as follows:

Myocardial performance after albumin solution exchange was sustained on right heart bypass in only one of ten animals. SFHS animals had a significant drop in stroke volume at 14 torr after exchange, but the 50% performance level could be sustained. Coronary blood flow rose and MVO_2 fell in all groups although the statistically non-significant mean differences were less with SFHS. The $S(a-cs)O_2$ Arterial-coronary sinus oxygen content difference fell significantly with albumin solution and nonsignificantly with SFHS. They concluded that although myocardial performance decreased with SFHS, they believed these comparative results support the use of SFHS at a hematocrit of 5%. The same comments made on the last paper pertain to this paper.

Paper 15. Kim HW, Rowley BA, Feola M, Roberts LA. 1983. Effect of stroma-free hemoglobin on an isolated perfused rabbit interventricular septum. [Abstract No 2766] Fed Proc 42:765.

Three different 7% stroma-free hemoglobin solutions were tested as potential perfusates for isolated perfused rabbit interventricular septa. Controlled activity of the septum, stimulated at approximately at 1.5 pps, was established for Tyrode buffer. After one hour equilibration with Tyrode medium, SFHS was perfused at a comparable flow rate and changes in developed tension, resting tension, $\pm$ dP/dT, and perfusion pressure were followed. After 20 min equilibration in SFHS, a 10-min stop flow was imposed followed by a 30-min reflow. One of the SFHS (Prep C) preparations, which was prepared by a sterile filtration technique, increased developed tension by 20% during the control period. In contrast, two other SFHS groups (A and B) preparations and Tyrode medium allowed recovery over 30 min of 63, 79, and 87% of developed tension respectively. The Prep C stroma-free solution allowed 95% recovery of control developed tension after 10 min stop flow. Vascular resistance as indicated by increased perfusion pressure increased with both SFHS and with a 7% albumin solution in Tyrode buffer. The increase perfusion pressure was minimal for the Prep C stroma-free solution compared to other stroma-free solutions. The Prep C stroma-free solution, which was prepared by a sterile filtration technique, did not cause any adverse effects on the septum and appears to improve contractile activity when used as a perfusion medium for this preparation.

Paper 16. Langdale LA, Gould SA, Lakshman RS, et al. 1983. Stroma-free hemoglobin - *a myocardial depressant?* [Abstract] J Trauma, 23:647.

Twelve adult baboons were anesthesized, intubated, and mechanically ventilated on room air. These baboons received atropine, phenobarbital, pavulon, and ketamine. No calcium was given. All animals were exchanged transfused with Dextran-70 to 10% hematocrit. The animals were then randomly assigned to receive autologous RBCs or an equivalent amount of stroma-free hemoglobin. By perfusion of the test solution, pulmonary capillary wedge pressure was maintained at baseline levels. Hemodynamic measurements were obtained at comparable total hemoglobin concentrations.

The cardiac output increased significantly with hemodilution in both groups and declined towards baseline with resuscitation. The cardiac outputs were not significantly different throughout the two groups in the study. The total study lasted 6 to 8 hours and hemoglobin solution was in the baboons for a total of 2 to 3 hours, minimum. There were no arrhythmias noted on continuous electrocardiographic monitoring. In conversation with Dr. Langdale, she told me that although the cardiac output in the group which received stroma-free hemoglobin did return to prehemodilution levels, there was an initially greater drop in the cardiac output of this group than in the group which received autologous RBCs. Although not significantly different, this drop indicated to her that there might be some suppression of cardiac output due to hemoglobin solutions. The authors concluded that the cardiac response to resuscitation with stroma-free hemoglobin is not different than that with RBC therapy. There was no evidence from myocardial depression with stroma-free hemoglobin transfusion.

Paper 17. Dennis RC, Vogel WM, Apstein CS, Valeri CR. 1983. Potent vasoconstrictor substance in stroma-free hemoglobin observed during perfusion of isolated rabbit hearts. [Abstract] Advances in Blood Substitute Research, Bolin, RB, Geyer RP, Nemo GJ, eds. Prog Clin Biol Res 122:427-428.

In this experiment, stroma-free hemoglobin solution from 5 to 200 mg/percent and modified Krebs buffer perfused at a rate of 7 mL/min/gram heart produced a dose related significant increase in coronary perfusion pressure from a normal value of 67 mL Hg to a peak increase of 88% over baseline at 200 mg% hemoglobin. This vasoconstriction was not explained by increased oxygen affinity of the stroma-free hemoglobin. The increased coronary vascular resistance may explain a myocardial depression observed in the dog, pig and baboon treated with stroma-free hemoglobin. My conversation with Dr. Dennis and Dr. Vogle at the Naval Blood Research Laboratory and Cardiac Muscle Research Laboratory at Boston University School of Medicine on 25 Oct 83 indicated that the research mentioned in this abstract had not progressed far beyond this stage and no publication of this material was anticipated for the next six months minimal.

Paper 18. Moores, WY, White FC, Bloor C, Greenburg AG, Mark R, Willford DC. 1982. The physiologic effects of oxygen transport by hemoglobin solutions. In: Advances in Blood Substitute Research, Bolin RB, Geyer RP, Nemo GJ, eds. Prog Clin Biol Res 122:89-99. And...

Paper 19. Moores, WY. 1983. Data Summary. Evaluation of stroma-free hemoglobin solutions as resuscitative fluids for the injured soldier. Number DAMD17-79C-9153.

These papers concern the effects of hemoglobin solutions on an acute right heart bypass preparation, a chronic exercising canine preparation and a chronic exercising swine preparation. The solutions used were 1) 7% bovine albumin solution, 2) stroma-free hemoglobin solution prepared by filtration and centrifugation and essentially a Rabiner preparation, and 3) a similarly prepared stroma-free hemoglobin solution modified with pyridoxal phosphate (Greenberg, 1977).

Acute right heart bypass model in the pig. This utilizes a methodology reported previously (Moores, J. Thoracic Cardiovasc Surg. 83:153-162, 1982). Measurements were taken before and after exchange transfusion resulting in a hemodilution of 50% of the control value. The P_{50} of the whole blood dropped significantly in both groups that received hemoglobin solutions but did not differ between the types of stroma-free hemoglobin solution. The stroke volume and percent segmental shortening of the myocardial tissue were assayed. Myocardial performance decreased significantly in all groups, and although the largest mean drop did occur in the albumin group, it was not possible to distinguish them statistically from the other groups. Mean arterial blood flow increased in all groups after exchange but was not significantly different between groups. Arterial oxygen content was significantly decreased after exchange in all groups, and the decrease in the albumin group was significantly different from the two hemoglobin solution groups. In spite of the differences, it was not possible to demonstrate significant differences in myocardial oxygen consumption, arterial coronary sinus oxygen content difference, or coronary sinus pH, either within or between groups. Note that all groups showed a decrease in the mean value for myocardial oxygen consumption, and arterial coronary sinus oxygen content difference, and the difference was greatest for the albumin group. All groups failed to demonstrate any persistent anaerobic metabolism as manifested by lactate production. Although the arterial oxygen content data supported the concept that transfusion with SFHS in a 50% exchange model did result in more oxygen availability for the myocardium, there was little advantage for myocardial performance between groups. Although there was an increased arterial oxygen content with stroma-free hemoglobin solutions, this did not result in

better coronary blood flow, myocardial oxygen consumption, arterial-coronary sinus oxygen content difference, coronary sinus pO_2, or lactate extraction. If there was a 50% volume change, then a resuscitative solution did not need to carry oxygen in order to be efficacious and support myocardial function.

The second part of the study used minimally instrumented dogs which were previously trained to run on a treadmill. After initial measurements, the animals were exchanged transfused while awake with either modified stroma-free hemoglobin solution, unmodified stroma-free hemoglobin solution or albumin. Thirty minutes after exchange all animals were exercised on a treadmill. Animals which received albumin had less exercise tolerance than those receiving hemoglobin solutions. This was manifested by decreased rate of exercise and decreased maximum tolerated time of exercise. The resting heart rate was elevated and the mean exercise heart rate was less for the albumin exchanged group compared with the other groups. The albumin animals also showed a significant elevation in the recovery heart rate compared to the others. All the animals which received stroma-free hemoglobin solution or albumin had a significant drop in arterial oxygen content; however, this was greatest in the albumin group. The AV oxygen content differences varies greatly between animals but tended to be better sustained in the groups which received Sham Operation. Similar results were obtained for venous pO_2. There was no advantage for the use of hemoglobin solutions. The animals which received albumin had the largest values for venous lactate both at rest and for moderate exercise, but the data were not statistically significant. At 24 hours and 7 days after the experiment, there was no difference between groups in their ability to tolerate exercise. Again as was the case for the acute swine model, the exercise dog model did not show an advantage to modifying hemoglobin solution to normalize the P_{50} value.

The third section of these studies concerned chronically instrumented pigs. Unmodified hemoglobin solution and albumin solution were tested. All pigs were exchanged with 2 liters of fluid to achieve a hematocrit value of 14%. In all situations, the hematocrit rose with exercise. After exchange with albumin, the cardiac output rose, whereas with stroma-free hemoglobin solution there was no change at rest in cardiac output. The increase in cardiac output after albumin injection was due to an increase in heart rate because the stroke volume remained unchanged. The animals in both groups showed a decrease of approximately 50% in exercise response following exchange, with a shorter time noted for exhaustion in both groups. There was no change between groups in stroke volume. The dP/dt increased significantly for hemoglobin solution but not for albumin following exercise, but with resting dP/dt value was higher for albumin. The diastolic diameter remained unchanged with exercise

in both groups, whereas left atrial pressure increased which indicated an increase in the pressure volume relationships in all animals subjected to exercise. The arterial-venous oxygen content difference was significantly lower for the albumin exchanged animals during both rest and exercise, whereas the arterial-venous oxygen content difference in the stroma-free hemoglobin group was similar to the normal response during the control period, both at rest and exercise. There was no difference for oxygen consumption for either group, although the mean value for oxygen consumption in the albumin group was lower than for the other group. Arterial lactate values went up in both groups with exercise and was significantly greatest in the albumin group. The blood flow studies did not reach statistical significance between the two groups; however, there was a trend to close approximation to control values with stroma-free hemoglobin solution as opposed to albumin solution. There was no difference in myocardial blood flow although the trend was more toward an abnormal increased flow both during exercise and at rest in the albumin group. In the case of brain blood flow, there was increased flow during exercise that reached statistical significance in the albumin group. Kidney, intestine, stomach, liver, and spleen blood flow all decreased during exercise in all groups and was more marked for the albumin group.

Albumin solution resulted in a hyper-dynamic performance of the heart at rest manifested by increased cardiac output and increased df/dt. The SFHS group did not accelerate myocardial performance until they were exercised. Presumably if the fluid used contains oxygen, then an increased cardiac performance is not necessary at rest for metabolic needs. The SFHS group consistently resulted in greater arterial-venous oxygen content difference. This is not reflected in a greater oxygen consumption though since albumin animals tended to compensate for the decreased arterial-venous oxygen content difference with an increase in cardiac output. The blood flow to organs such as the heart and skeletal muscle increased with exercise, but blood flow to the viscera decreased with exercise, and the response was more marked with albumin than with stroma-free hemoglobin, and a more normal response was obtained with stroma-free hemoglobin solutions.

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